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Addressing Association Entropy by Reconstructing Guanidinium Anchor Groups for Anion Binding: Design, Synthesis, and Host–Guest Binding Studies in Polar and Protic Solutions

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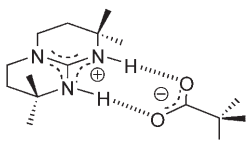
Abstract: The bicyclic hexahydropyrimidino[1,2*a*]pyrimidine cationic scaffold has a well-known capacity to bind a variety of oxoanions (phosphates, carboxylates, squarates, phosphinates). Based on this feature, the parent host was supplemented with *sec*-carboxamido substituents to generate compounds **1**–**3** in an effort to improve the anion-binding affinity and selectivity and to learn about the role and magnitude of entropic factors. Bicyclic guanidinium compounds were prepared by a convergent strategy via the corresponding tetraester **22** followed by catalytic amidation. Host–guest binding studies with isothermal titration calorimetry in acetonitrile probed the behavior of artificial hosts **1**–**3** in comparison with the tetraallylguanidinium compound **4** on binding *p*-nitrobenzoate, dihydrogenphosphate, and 2,2′-bisphenolcyclophosphate guests that showed enhanced affinities in the 10^5–$10^6\,M^{-1}$ range. Contrary to expectation, better binding emerges from more positive association entropies rather than from stronger enthalpic interactions (hydrogen bonding). In an NMR spectroscopy titration in DMSO, *o*-phthalate was sufficiently basic to abstract a proton from the guanidinium function, as confirmed by an X-ray crystal structure of the product. The novel carboxamide-appended anchor groups also bind carboxylates and phosphates, but not hydrogen sulfate in methanol with affinities in excess of $10^4\,M^{-1}$. The energetic signature of the complexation in methanol is inverted with respect to acetonitrile solvent and shows a pattern of general ion pairing with strong positive entropies overcompensating endothermic binding enthalpies. This study provides an example of the fact that bona fide decoration of a parent guanidinium anchor function with an additional binding functionality may provide the desired enhancement of the host–guest affinity, yet for a different reason than that implemented by design as guided by standard molecular modeling.

Keywords: anion recognition · entropy · guanidinium · host–guest systems · isothermal titration calorimetry

Introduction

Cationic guanidinium functions play prominent roles in molecular recognition of oxoanionic species in both the living world and in abiotic applications.[1–5] In biology they are frequently involved in anion binding to proteins[6] as a consequence of their presence in the side chain of the ubiquitous proteinogenic amino acid arginine. The structural definition of the arginine–oxoanion interaction covers a broad range—from spatially quite relaxed ion pairing (e.g., in the coiling of DNA around the basic histone protein core of nucleosomes[7]) to a very dedicated geometric correspondence of the two moieties, as sketched in Scheme 1 (e.g., in the recognition of C-terminal carboxylate by carboxypeptidase A).[8]) The latter motif, in particular, inspired the use of this pattern in artificial receptors,[9–22] although such a well-defined structural relationship cannot be taken for granted. For most instrumental methods, it is indistinguishable from a structurally relaxed and dynamically disordered, yet time-averaged preference for the depicted association mode. Distinction between the two extreme binding modes is of paramount importance for the functional utility of the anchor group and is thus



Scheme 1. A sketch of the guanidinium oxoanion binding motif as it occurs in many natural and artificial receptors.

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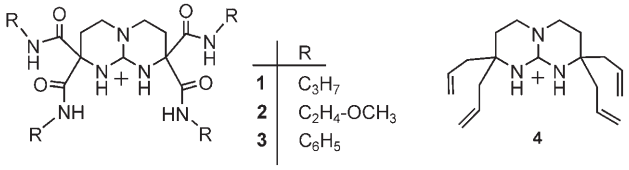
indispensable in the rational design of artificial guanidinium receptors.

Regrettably, there is no immediate and unambiguous analytical tool available to report on the geometrical fuzziness of host–guest interactions in general. The diversity of binding modes, however, must be reflected in the entropy component of the association energetics because the respective configurational entropy contributes a substantial share to the overall entropy output. Even though the entropy of association is now readily accessible by modern calorimetry techniques, unfolding of the configurational component is less straightforward. At best, a qualitative estimate can be deduced from a comparison of closely related host–guest pairs measured under strictly identical environmental conditions (solvent, solution composition, temperature, etc.) to minimize interference from unspecific solvation effects. Such a trend analysis may then reveal a qualitative picture of the variation of static and dynamic disorder in the host–guest complex with incremental structural modification.

The parent guanidinium receptor depicted in Scheme 1 offers the opportunity to evaluate a structure–binding mode correlation in a popular supramolecular binding motif of proven utility. This abiotic host–guest system features a robust and easy-to-grasp recognition pattern that is unperturbed by remote influences endemic to the much more complex natural pendants. The trend analysis of the energetics in a series of rather rudimentary molecular recognition motifs should provide an experimental probe to address the basic role of entropy in molecular recognition and its potential for supramolecular design.

Towards this goal, appending the 6,6-membered bicyclic guanidinium scaffold with *sec*-carboxamide moieties in the α,α' positions to give guanidinium hosts **1**–**3** was deemed an optimal choice because *sec*-carboxamides are well-established anchor groups in anion binding[23–27] and their geminal placement in the immediate vicinity of the binding site was



expected to enforce its stickiness towards the anionic guests by the sheer accumulation of hydrogen-bond donor functions and the suboptimal solvation of the individual groups as a result of overcrowding.

We have already reported an example that followed this design outline.[28] The binding of 6,6-gua-carboxamide **1** to a series of oxoanions revealed the amazing fact that guest affinity in acetonitrile was indeed enhanced in **1** relative to an analogous guanidinium host **4** that lacked the carboxamide moieties; however, for a different reason than originally anticipated: for all guest anions tested, the attractive enthalpy of association was diminished and the higher affinity was exclusively due to a much more positive association entropy component. From a consideration of the main factors contributing to this surprising observation, a rationalization was advanced that the deeper cause of this unexpected result could be attributed the increase in configurational entropy of the binding partners as a consequence of the low structural definition in the host–guest complex. Herein we report the design, synthesis, and host–guest binding of two more candidates (**2** and **3**) of the guanidinium carboxamide variety that stand out by their host–guest affinity, even in polar protic solutions (K_{ass} up to $37000\,M^{-1}$ in methanol). In addition, an unprecedented transprotonation by the *o*-phthalate dianion was found that supports calls for caution voiced recently with respect to anion binding to artificial amide receptors.[29]

Results and Discussion

Planning and synthesis: Before defining the guanidinium carboxamides as synthetic targets, molecular modeling was carried out to confirm their suitability. Geometric optimization of the benzoate salt of anilide **3** in vacuo initially converged on a structure (Figure 1a) that placed the guest anion almost juxtaposed to the guanidinium unit in the familiar salt-bridge/hydrogen-bond pattern depicted in Scheme 1. This motif was supplemented by two further hydrogen bonds donated from a carboxamide on either the left- or right-hand side of the bicyclic molecule. The two remaining carboxamides were engaged in lateral amide–amide interactions to form 6-membered hydrogen-bonded rings. Subjecting this ensemble to a molecular dynamics run over 20 ps followed by simulated annealing produced a significant structural change. Now the guest carboxylate group was held by two hydrogen bonds to the guanidinium cation and one carboxamide group in a slightly dissymmetric fashion, while two amides again formed hydrogen-bonded rings; however, one of them spans the bicyclic frame to form a supramolecular macrocycle (Figure 1b). One carboxamide

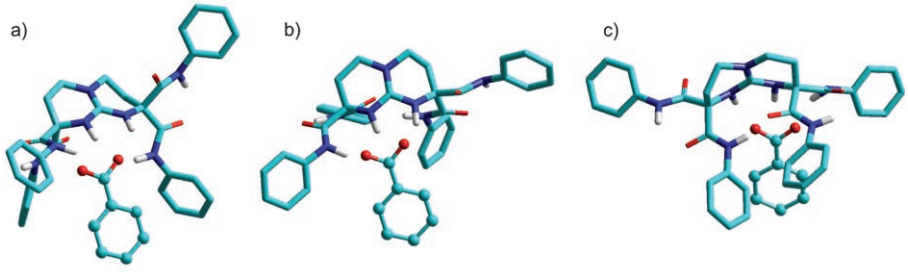
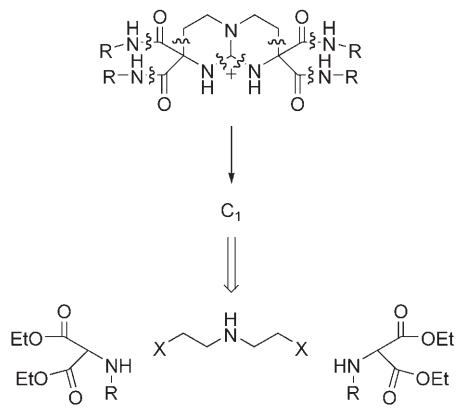


Figure 1. Energy-minimized structure of the benzoate salt of anilide **3** by using the Amber force field a) in vacuo, b) after a molecular dynamics run for 20 ps and simulated annealing, and c) rear view of structure b.

was released from the network of hydrogen bonds and this pattern remained untouched when the complex was embedded and energy-minimized under periodic boundary conditions in a water box containing more than 5800 water molecules.

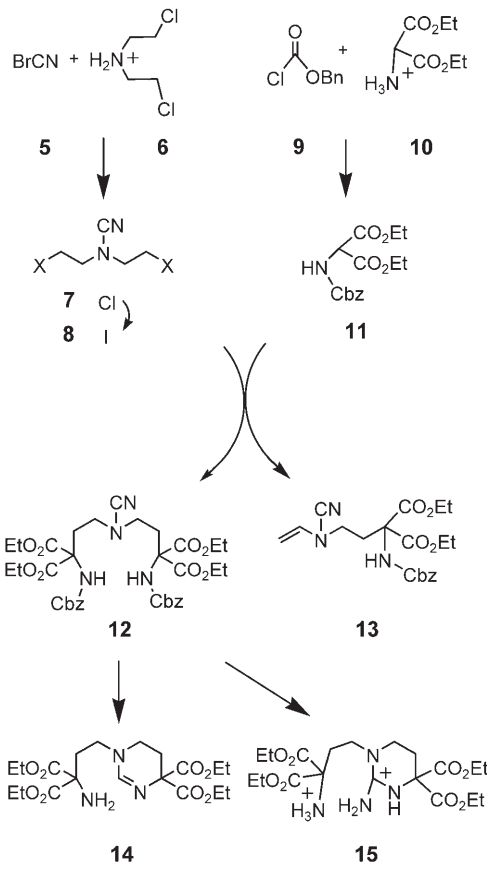
The modeling results support our initial idea that the carboxamido functions help in guest binding. Even though not all potential hydrogen-bond donors were found to simultaneously interact with the bound anion in the minimum-energy structure, they stitch together a network of hydrogen bonds. This organizes the entire host and thus aids host–guest binding by limiting the entropic loss expected on merging the binding partners into an associated structure. On this basis, guanidinium compounds **1**–**3** were judged to be promising synthetic goals. As suggested by their symmetric topology, a convergent strategy, such as that shown in Scheme 2, appeared to be the most efficient for their synthesis. Apart from the final standard conversion of an ester into an amido group, our approach takes advantage of the combination of two building blocks, **8** and **11**, that are simple derivatives of cheap commercial starting materials.



Scheme 2. Retrosynthetic approach to compounds **1**–**3**.

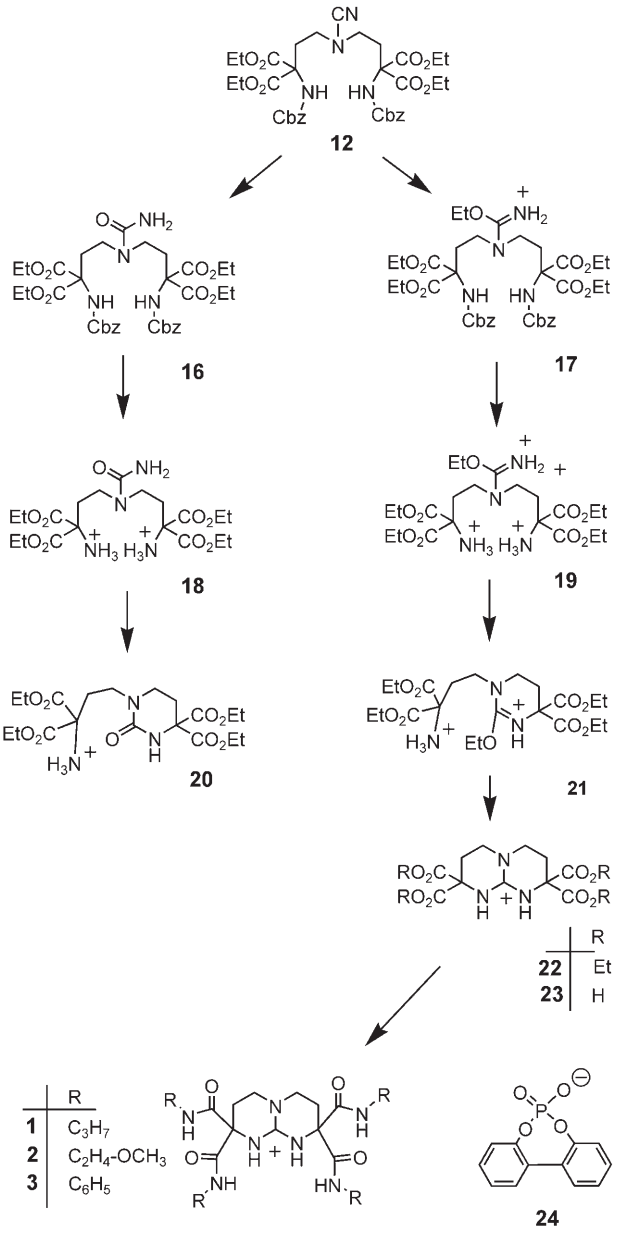
The synthesis of guanidinio carboxamides **1**–**3** followed the route depicted in Schemes 3 and 4. The alkylative C–C coupling of building blocks **8**[28] and **11**,[30] which were obtained by short literature procedures, required low reaction temperatures ($<10\,^{\circ}C$) for several days to give a yield of $>70\,\%$ of key intermediate **12** and to avoid substantial loss due to the formation of elimination product **13**. A 20:1 preference for the desired product was achieved under the specified conditions, whereas raising the temperature to $50\,^{\circ}C$ produced a 1:1 mixture of these compounds.

Originally, deprotection of the amino functions by hydrogenation in the next step was presumed to directly furnish the guanidinium monocycle **15**, which seemed to be an attractive candidate for ring closure to the bicyclic skeleton. Instead, with the Pd/carbon catalysts tested, the product isolated in almost quantitative yield was monocyclic formamidine **14**, which undoubtedly arose from preferential hydrogenation of the cyanamido function followed by monocyclization taking one deprotected amino group as the nucleophile. This outcome did not change when more drastic conditions in transfer hydrogenation were employed (Pd/C, cyclohexadiene, reflux) or by the use of trimethylsilyliodide as a demasking agent. Strong acid solvolysis (HBr/propionic acid), however, changed the course of the reaction and yielded monocyclic guanidinium salt **15** as the main product. Unfortunately, this compound resisted all attempts to produce the desired bicyclic structure by amine exchange of the guanidinium unit.[31] We envisaged an escape from this dead-end road to be the elaboration of the cyanamido function, which was introduced at the very start to serve as an amino protecting group as well as a synthon for the core guanidino carbon, into a more reactive moiety susceptible to attack by the shielded carbinylamino atom. Treatment of cyanoamide **12** with dilute sulfuric acid cleanly gave the corresponding urea **16**, which in turn could be deprotected by catalytic hydrogenation to give **18** in quantitative yield. The putatively simple monocyclization to cyclic urea **20** presented a major obstacle, again testifying to the low nucleophilicity of the carbinylamino group. After some optimization, compound **20** was obtained in a moderate yield of 27 %, which suggested that we should look for a better alternative.



Scheme 3. Cbz = carbobenzyloxy

A viable alternative was to subject **12** to the Pinner reaction to afford isourea **17** as the initial step in a sequence that was profitably conducted without purification of the in-



Scheme 4.

termediates. Thus, imidate **17** was hydrogenated and cautiously neutralized to give bicyclic guanidinium tetraester **22** in a one-pot reaction in a yield of 72% over four steps. The ester-to-amide transformation was initially attempted by using the classic method of hydrolysis to give acid **23** followed by amidation after activation to the acid chloride or a mixed anhydride. This stage, however, proved intractable owing to the insolubility of the starting acid. Direct transamidation of ester **22** with the respective amines by using promotion by alkylaluminium reagents proved effective.[32,33] The acidity of the substrate and the alcohol formed required a considerable excess of the organometallic Lewis acid, yet target compounds **1**–**3** were isolated from the multistep sequence in a yield of 70–80% as crystalline salts. The assigned structures are fully supported by single-crystal X-ray analyses of the halide salts (see the Supporting Information), which also show the peculiar hydrogen-bonding ring formation within the malonic amide moieties that was predicted by the modeling studies. The occurrence of this motif in the solid state in two compounds attests to the stability of this pattern. In conjunction with the computational results, there is now reason to presume that this kind of host preorganization will also persist in solution. All of the binding studies used iodide as the counteranion because these salts are readily purified and this counterion does not interfere with oxoanion complexation.[12]

Binding studies: Initial NMR spectroscopy binding studies of propylamido host **1** and dihydrogenphosphate in acetonitrile had revealed a saturation isotherm when using the amide NH signal as a probe.[28] However, a significant nonrandom swing of the experimental data points across the fitted hyperbola describing the 1:1 stoichiometric model was interpreted (and later confirmed by isothermal calorimetry (ITC) measurements) as an indication of higher-order complex formation. To avoid such risk and further escape any restrictions set by insufficient solubility of the host and guest salts, the solvent was switched to DMSO. Titration of anilide host **3** with *o*-phthalate dianion (tetraethylammonium salt) in this solvent, however, did not show the anticipated shift of the NH signals characteristic of rapid equilibration-averaged host–guest binding (Figure 2).

Instead, the NH signals of the amido and guanidinium groups broadened and eventually disappeared, but both kept their positions, indicative of a slow exchange process on the NMR spectroscopy timescale. The aromatic resonances of either salt were unaffected at host–guest molar ratios below 1:1, whereas the methylene signals of the 6-membered rings were gradually shifted upfield (Figure 2). Regrettably, this change could not be quantified because the host signals hide behind the solvent and guest resonances for a considerable proportion of the titration. At host–guest molar ratios greater than 1:1, substantial redistribution of the aromatic signals was seen (Figure 2d, e) and was deconvoluted by means of a COSY 2D spectrum into two sets of coupling networks. The relative proportion of these sets did not depend on the concentration of the added guest, which suggests that they are not derived from supramolecular association.

A tentative explanation was gleaned from the serendipitous crystallization of such a titration mixture from methanol. Contrary to expectation, the X-ray analysis of the crystals did not reveal the presence of a guest anion, but corresponded to the free base form of host **3**, as depicted in Figure 3. The structure shows a similar hydrogen-bonding pattern at one geminal dicarboxamide side, as that observed in guanidinium salt **3** (see the Supporting Information). The other malonic amide moiety, however, is no longer fixed in a mutual hydrogen-bond interaction, but contains the NH vectors pointing towards the proximal nitrogen atom of the guanidine (NH···N $\approx$2.1 Å). The free base nature is clearly

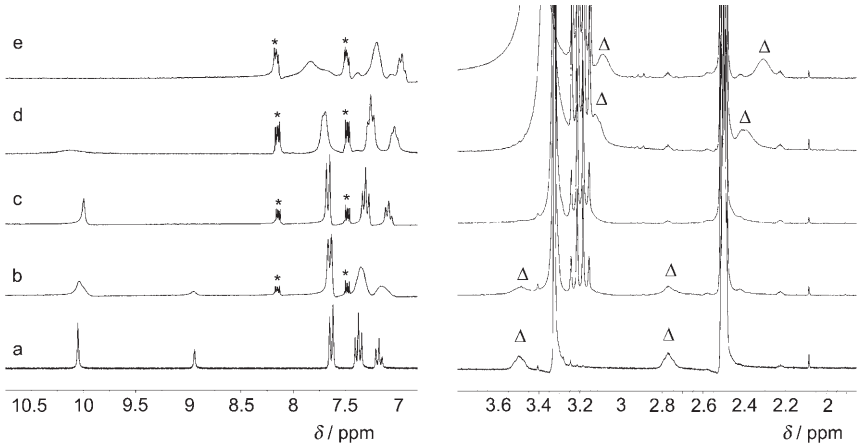


Figure 2. ^{1}H NMR spectra ([D$_6$]DMSO, 250 MHz) recorded for the complex formed by the anilide **3** (2 mM) with a) 0 mM, b) 1.2 mM, c) 2.4 mM, d) 3.6 mM, and e) 4.8 mM of *o*-phthalate. The designated signals arise from the guest anion (*) and methylene groups (△) of the 6-membered rings in the anilide host **3**.

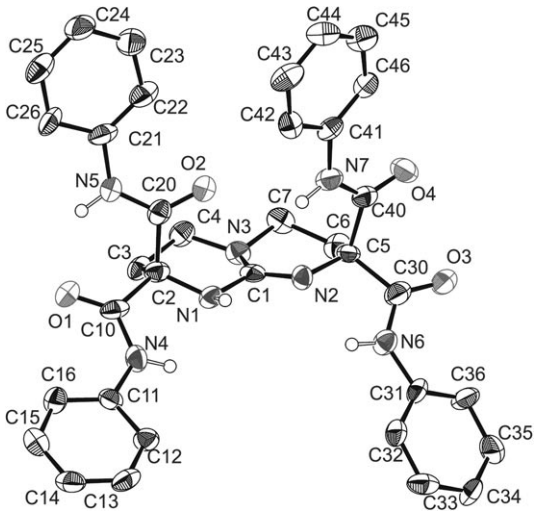


Figure 3. ORTEP-style plot of anilide **3** showing the free base form (d_{C1-N2} = 1.29 Å; d_{C1-N1} = 1.40 Å).[37] Thermal ellipsoids are drawn at the 50% probability level.

reflected by a much shorter C–N distance in the proximal ring (≈1.29 Å versus 1.40 Å in the other ring), indicating double bond character, and the pronounced pyramidalized configuration of the nitrogen atom in the distal ring. Clearly, the basicity of the phthalate dianion in the organic solvent, probably owing to a short and strong hydrogen bond connecting the carboxylate moieties in the monoanion, suffices to deprotonate the guanidinium cation,[34] particularly if the free base form of the host is stabilized by hydrogen bonding to the adjacent carboxamides. Such deprotonation is impossible with all of the other oxoanions used in this study (see below) owing to their much lower basicities. The molecular process occurring during the titration in DMSO thus appears to be a transprotonation rather than a host–guest interaction. The two sets of observed aromatic proton signals most probably refer to the respective malonic amide substructures adjacent to or remote from the C=N double bond in the guanidine. Similar switching between genuine supramolecular association or covalent proton transfer has frequently been found with the fluoride anion,[29,35] which also constitutes an extremely basic species in organic solutions.[36]

Previous investigations of the energetics and speciation of guanidinium phosphate host–guest binding revealed an advantage of ITC over NMR spectroscopy for analysis in this peculiar case.[28] Following up on these studies, host compounds **2** and **3** were probed by *p*-nitrobenzoate, phosphate, and cyclic phosphate diester **24** in acetonitrile to assess the consequences and possible generalizations for the design of guanidinium anchor groups. The recurring feature in these interactions is the tendency to form complexes beyond 1:1 stoichiometries. An illustrative example is depicted in Figure 4 for the interaction of **3** with nitrobenzoate, which shows strong deviations from the response expected for a 1:1 binding model in the regimes of excess host over guest (i.e., at molar ratios <1 at the beginning of the titration) as well as at higher guest-to-host molar ratios. The analysis of such systems is greatly facilitated when experimental conditions are found that allow simplification of the interaction model. This is shown in Figure 4b as an example in which dilution of the starting concentration largely suppressed the initial formation of higher-order host–guest complexes. Under this precondition, the deconvolution of the macroscopic heat response into individual components is possible, as conveyed by the fitted curve which represents the experimental data quite well (Figure 4). The conditional association constants collected in Tables 1 and 2 were derived on this basis.

In general, the pattern observed with hosts **2** and **3** greatly resembles the results obtained earlier with propylamido host **1** binding the same guest.[28] Relative to tetraallylguanidinium host **4**, which displays clean 1:1 binding, all carboxamido hosts **1–3** possess a higher affinity for these guests; however, not as a result of more attractive binding (which was the original foundation of the design concept), but for a more positive output in the association entropy.[28] The only exception to this rule is the binding of anilide host **3** to dihydrogenphosphate. Although in all cases this guest shows the most negative interaction enthalpy among the oxoanions, which most probably reflects the greater potential for hydrogen bonding because of its additional hydrogen-bond donor capabilities, anilido host **3** stands out for its special energetic signature. Host **3** is the only anchor group in this series that provides a negative entropy contribution that counteracts a dramatically enhanced attractive enthalpy of binding. On

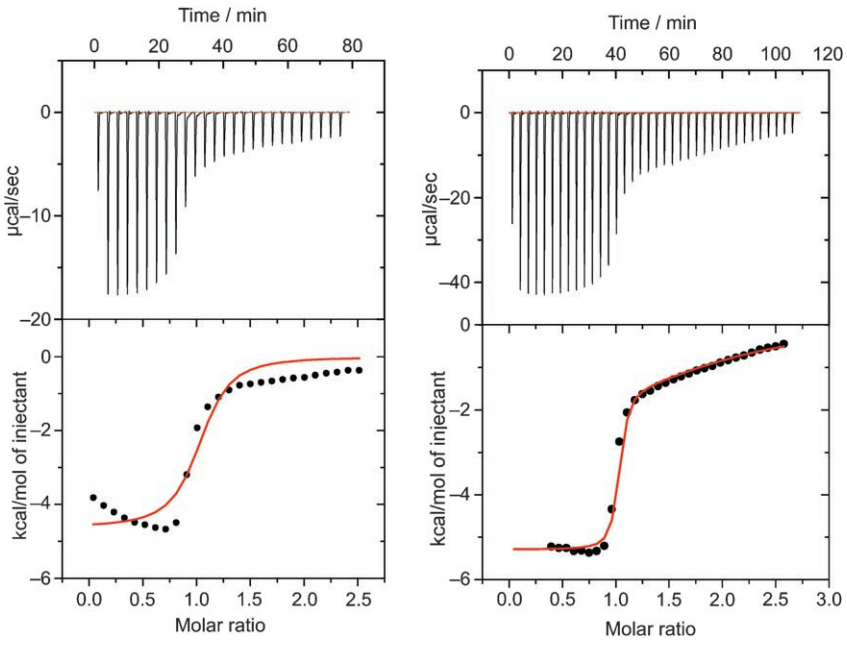


Figure 4. ITC traces of the titration of *p*-nitrobenzoate into the solution of host **3** in acetonitrile at 303 K a) at 4.33 mM and b) at 1.3 mM. The solid lines represent fitting curves on the basis of a 1:1 binding model (left) or sequential 2:1 model (right).

the balance sheet, this leaves a smaller affinity than observed in any other host–guest combination; however, for the benefit of a better structured complex.[38,39] Undoubtedly, the organizing role of the phosphate guest must be synergistically supported by mutual interactions of the phenyl rings to explain this result.

The respectable affinity found for these host–guest pairs in the polar, yet non-protic acetonitrile solvent fostered the expectation that association might also persist in protic solvents. ITC titrations performed with anilide host **3** in methanol did indeed corroborate this presumption (Table 2). The energetic signatures are in contrast with the results obtained in acetonitrile. In methanol, all associations are endothermic (except for one step in the binding of *p*-nitrobenzoate) and owe their affinity to an excessive positive entropy contribution. Thus, they resemble the general ion-pairing process that features substantial desolvation of both ionic partners without generating a singular host–guest complex structure. Disregarding hydrogen sulfate, which did not produce a sufficient heat effect to allow analysis, the other guests showed unambiguous supramolecular binding behavior.

Table 1. 1:1 Host–guest binding energetics of guanidinium anchor groups (iodide salts) with oxoanions in acetonitrile at 303 K.

	TBA *p*-nitrobenzoate				TBA H_2PO_4				TBA 2,2′-bisphenolcyclophosphate **24**			
	K_{ass} [M^{-1}]	$-\Delta G°$ [kJ mol^{-1}]	$-\Delta H°$ [kJ mol^{-1}]	$T\Delta S°$ [kJ mol^{-1}]	K_{ass} [M^{-1}]	$-\Delta G°$ [kJ mol^{-1}]	$-\Delta H°$ [kJ mol^{-1}]	$T\Delta S°$ [kJ mol^{-1}]	K_{ass} [M^{-1}]	$-\Delta G°$ [kJ mol^{-1}]	$-\Delta H°$ [kJ mol^{-1}]	$T\Delta S°$ [kJ mol^{-1}]
1	2.0×10^{-5}	30.8	17.6	+13.2	1.3×10^{6}	35.6	17.5	+18.0	4.8×10^{4}	27.2	10.3	+16.8
2	9.3×10^{-4}	28.8	13.2	+15.6	1.6×10^{6}	36.1	12.9	+23.2	1.7×10^{4}	24.4	9.9	+14.6
3	1.2×10^{-6}	35.5	22.1	+13.1	3.8×10^{4}	26.5	41.9	−15.4	2.7×10^{5}	31.6	12.1	+19.4
4	7.1×10^{-4}	28.2	22.3	+5.9	2.3×10^{4}	24.6	23.4	+1.1	1.9×10^{4}	24.7	14.7	+10.0

Table 2. Energetics of oxoanion binding (tetraethylammonium/tetrabutylammonium salts) to anilide **3** (as the iodide salt) in methanol at 303 K. The stoichiometry number *n* refers to the guest/host molar ratio as the experimentally determined fit parameter in a stepwise equilibrium system.

Entry	Guest	Model[a]	K_{ass} [M^{-1}]	$\Delta G°$ [kJ mol^{-1}]	$\Delta H°$ [kJ mol^{-1}]	$T\Delta S°$ [kJ mol^{-1}]
1	TBA benzoate	A	$K_1=180.3$	$\Delta G_1=-12.9$	$\Delta H_1=29.9$	42.9
			$K_2=3846$	$\Delta G_2=-20.5$	$\Delta H_2=49.4$	70.1
2	TBA-*p*-nitrobenzoate	A	$K_1=112$	$\Delta G_1=-11.7$	$\Delta H_1=-67.5$	−55.7
			$K_2=1.7\times10^{4}$	$\Delta G_2=-24.2$	$\Delta H_2=88.0$	112.2
4	TBA H_2PO_4	A	$K_1=96.7$	$\Delta G_1=-11.3$	$\Delta H_1=6.7$	18.1
			$K_2=5968$	$\Delta G_2=-21.5$	$\Delta H_2=4.8$	26.2
4	TEA naphthalene dicarboxylate	A	$K_1=2192$	$\Delta G_1=-19.1$	$\Delta H_1=3.0$	22.0
			$K_2=1.95\times10^{4}$	$\Delta G_2=-24.5$	$\Delta H_2=65.7$	90.3
5	TEA phthalate	B	3.3×10^{4}	−25.0	45.2	70.2
6	TEA terephthalate	B, $n=0.35$	1.45×10^{4}	−22.1	90.3	112.4
7	TEA HSO_4			insufficient heat response		
8	TEA oxalate	B, $n=0.57$	3.7×10^{4}	−25.0	60.6	85.6
9	TEA fumarate	B, $n=0.4$	8441	−21.7	38.0	59.8
10	TEA isophthalate	B, $n=0.45$	1.7×10^{4}	−24.1	24.5	48.6
11	TEA squarate	B, $n=0.5$	4077	−20.1	34.2	54.2

[a] A = titration mode: guest into host solution; 2 sequential-site model; ligand-in-cell, B = titration mode: guest into host solution; one-site model.

Except for phthalate, which adhered to a 1:1 stoichiometric model, the heat responses of the other anions required more complicated binding models to reproduce the enthalpy output. In some cases (Table 2 entries 6–11), the individual steps could not be deconvoluted; however, the stoichiometric factor clearly indicated the involvement of two binding events representing the association of one and two guanidinium anchor groups onto the bis-anionic guest. Charge matching is an important, yet not the exclusive cause for higher complex formation. Also, in the case of monoanionic guests the energetic profile cannot be fitted without the participation of higher-order complexes. However, the absolute affinities are somewhat smaller than those of the dianions. Nevertheless, host–guest association between singly charged partners in a protic solution reaches quite an impressive level ($K_{ass} = 10^4\,M^{-1}$), despite the simple construction blueprint of this anchor function. Selectivity is poor (about a factor of 10), but may be improved by implementing restrictions affecting the accessibility and conformational flexibility of the binding site. A sufficient amount of binding free energy in polar solvents, as secured in this study, is a necessary prerequisite to allow tuning of the various design options.

Conclusion

With the goal of learning about the role of entropy in molecular recognition, the elaboration of the prominent bicyclic guanidinium anchor group for oxoanions was undertaken by supplementation of the parent moiety with *sec*-carboxamido groups. X-ray crystal structures confirmed the expectation emerging from modeling studies that the variety of conformations is limited by the build-up of an intramolecular hydrogen-bonded network. The resulting structural organization predisposes the guanidinium host for guest binding using only a fraction of the formally available hydrogen-bond donors and allows rationalization of an unexpected transprotonation occurring with the most basic phthalate guest. Binding studies by ITC titrations reveal high affinity in acetonitrile that exceeds ordinary ion pairing between monoionic partners by a hundred thousand fold.[40] The affinities are somewhat diminished in methanol ($K_{ass} = 10^3$–$10^4\,M^{-1}$), but more importantly show an energetic profile that is unlikely to emerge from geometrically dedicated complex formation. In all of the cases studied, entropy contributes a substantial share to the free energy of molecular recognition and even marks the dominant role in the protic solvent methanol. This experimental fact calls the validity of exclusively enthalpy-based design approaches into question.

Experimental Section

All of the experiments in organic solvents were performed under a nitrogen atmosphere and monitored by HPLC. The HPLC analyses were performed on a Merck-Hitachi instrument L 6200A or 655 A-11 pump connected to a Knauer L 4250 UV detector, a SEDEX 55 light-scattering detector, and a Kipps&Zonen two-channel recorder. The columns used in HPLC analysis were Phenomenex, Aqua C_{18}, 250×4.60 mm, 5 μ column, and Nucleodur-100-5 C_8 ec column. The solvents were purchased in pure analytical grade and distilled before use except for DMF, which was purchased in anhydrous quality from Aldrich, and acetonitrile, which was purchased in HPLC quality from Baker. Aqueous solutions were prepared from deionized, glass distilled water. The solvents (CH_2Cl_2, CH_3CN) were dried by passing them through a small column of activated alumina directly into the reaction vessel. All other chemicals were purchased in reagent quality from commercial sources and used as received. ^1H and ^{13}C NMR spectra were recorded on either a Brucker AC 360 (MHz) or 250 (MHz) instrument and were referenced with respect to the residual solvent peak. All of the melting points were measured in open capillary tubes by using a Fisher–Jones apparatus. Mass spectra were obtained on a Finnigan LQC: electrospray ionization (ESI, HPLC-MS) instrument. HRMS spectra were obtained on a Bruker microTOF-Q instrument. Elemental analyses were carried out by the microanalytical laboratory of the TU Munich. Calorimetric titrations were performed on the Isothermal Titration Calorimeter MCS-ITC from Microcal (USA). Molecular modeling was performed on Pentium PCs with HyperChem 8 molecular-modeling software from Hypercube. X-ray crystal structure analyses were carried out by the Inorganic Chemistry Department of TU Munich.

Bis(2-iodoethyl)cyanamide (8): Bis(2-chloroethyl)amine hydrochloride (**6**, 17.9 g,100 mmol) was dissolved in a mixture of water (50 mL) and CH_2Cl_2 (100 mL). The mixture was cooled to 0 °C and 4 N aqueous NaOH (25 mL) was added. A solution of **5** (13.1 g, 125 mmol) in CH_2Cl_2 (to make 30 mL) and 4 N aqueous NaOH (30 mL) were added alternately portionwise with vigorous stirring while keeping the temperature below 5 °C. The reaction mixture was then stirred for another 30 min at RT. The two phases were separated. The organic phase was washed with dilute acetic acid and then with brine, dried over magnesium sulfate, and the solvent was evaporated in vacuo to obtain a liquid residue that was distilled in a Kugelrohr apparatus (130 °C/0.05 torr) to afford **7** as a colorless viscous liquid (11.65 g, 70 %). This liquid (11.65 g, 70 mmol) was dissolved in acetone (50 mL) to which finely powdered NaI (30 g, 200 mmol) was added, and the mixture was heated at reflux for 3 d. After complete conversion to **8**, as seen from the HPLC analysis, the mixture was evaporated in vacuo. The resulting residue was redissolved in toluene and filtered. The filtrate was passed through a pad of alumina and then concentrated and dried under a high vacuum to afford **8** as a viscous colorless liquid (18.13 g, 74 %). HPLC analysis: $R_v = 10$ mL, Nucleodure-100–5 C_8 ec column, UV_{220}, flow = 1 mL min^{-1}, gradient from 10 % CH_3OH to 50 % CH_3OH in 10 min and then 50 % CH_3OH to 90 % CH_3OH in next 10 min, 0.1 % TFA; ^1H NMR (360 MHz, $CDCl_3$, 25 °C): $\delta = 3.45$ (t, $J = 7.0$ Hz, 4H; -N-C**H$_2$**), 3.29 (t, $J = 7.0$ Hz, 4H; -C**H$_2$**I); ^{13}C NMR (90.56 MHz, $CDCl_3$, 25 °C): $\delta = 115.1$ (-**C**N), 53.75 (-N-**C**H$_2$), −0.7 (-**C**H$_2$I).

Benzyloxycarbonylamino malonic acid diethyl ester (11): Diethyl 2-aminomalonate hydrochloride (**10**, 21.2 g, 100 mmol) and bis(trimethylsilyl) acetamide (BTSA; 24.8 mL, 100 mmol) were taken up in anhydrous diethyl ether (200 mL) under an inert atmosphere. The reaction mixture was cooled to 0 °C in an ice bath and **9** (14.4 mL, 100 mmol) was rapidly added. After addition was complete, another batch of BTSA (24.8 mL, 100 mmol) was added. The resulting cloudy reaction mixture was then allowed to warm slowly to RT and stirred for 30 min. On completion of the reaction, as monitored by HPLC analysis, the reaction mixture was washed with 0.1 N aqueous HCl (100 mL), dried over magnesium sulfate, and then evaporated in a rotary evaporator to give a gummy residue that was recrystallized from hexane/ether (1:1) to afford **11** as a white solid (26.5 g, 85 %). HPLC analysis: $R_v = 13$ mL, Phenomenex, Aqua C_{18}, 250×4.6 mm, 5 μ column, UV_{220}, flow = 1 mL min^{-1}, gradient from 50 % CH_3OH to 90 % CH_3OH in 10 min and then 90 % CH_3OH for a further 5 min, 0.1 % TFA; m.p. 30–32 °C (hexane/ether), (lit.[41] m.p. 32–33 °C); ^1H NMR (360 MHz, $CDCl_3$): $\delta = 7.36$ (s, 5H; aromatic-**H**), 5.85 (d, J = 6.8 Hz, 1H; -N**H**), 5.14 (s, 2H; PhC**H$_2$**O-), 5.03 (d, $J = 7.4$ Hz, 1H; -C**H**-N), 4.29 (q, $J = 6.5$ Hz, 4H; -OC**H$_2$**CH$_3$), 1.32 (t, $J = 7.0$ Hz, 6H; -OCH$_2$C**H$_3$**); ^{13}C NMR (90.56 MHz; $CDCl_3$): $\delta = 166.3$ (-**C**O, ester), 155.4

(-N**C**O-), 135.9, 128.5, 128.2, 128.1 (aromatic carbons), 67.3 (Ph**C**H$_2$O-), 62.6 (-O**C**H$_2$CH$_3$), 57.7 (-**C**H-), 13.9 (-OCH$_2$**C**H$_3$); MS: *m*/*z* (%): 310.2 (100) [*M*+H]$^+$.

***N*,*N*-Bis(3-benzyloxycarbonylamino-3,3-diethoxycarbonyl)propyl-1-cyanamide (12)**: Carbamate **11** (26.48 g, 85.7 mmol) in dry DMF (60 mL) was added to a suspension of NaH (3.59 g, 90 mmol) (60% dispersion in mineral oil) in dry DMF (20 mL) cooled to 5°C. The rate of addition was such that the temperature of the reaction mixture was maintained below 12°C (increase in temperature results in self-condensation of the ester). When the evolution of H_2 ceased and the reaction mixture became clear, a solution of **8** (10 g, 28.5 mmol) in DMF (30 mL) was added in two portions, half of the solution was added by syringe pump over a period of 6 h and the remaining half was added after stirring for 24 h (it is important to maintain the temperature of the reaction mixture below 20°C throughout the reaction time to avoid formation of the unwanted elimination product **13**). After complete addition of **8**, stirring was continued for 3 d, then the reaction mixture was poured into cold water containing 1 mL of acetic acid. The aqueous layer was extracted with CH_2Cl_2. The organic phase was washed several times with water, dried over magnesium sulfate, and concentrated in vacuo to give gummy substance that was further dried in a high vacuum to remove residual DMF. Recrystallization from ether/hexane (2:1) afforded **12** as a white crystalline compound (14.2 g, 72%). HPLC analysis: R_v=16.4 mL, Phenomenex, Aqua C_{18}, 250×4.60 mm, 5 μ column, UV_{220}, flow=1 mL min^{-1}, gradient from 50% CH_3OH to 90% CH_3OH in 10 min and then 90% CH_3OH for a further 5 min, 0.1% TFA; m.p. 72–73°C (ether/hexane); ^{1}H NMR (360 MHz, $CDCl_3$, 25°C): δ=7.34 (s, 10H; aromatic), 6.18 (s, 2H; -N**H**), 5.09 (s, 4H; PhC**H$_2$**O-), 4.24 (m, 8H; -OC**H$_2$**CH$_3$), 2.93 (t, *J*=6.5 Hz, 4H; -NC**H$_2$**-), 2.63 (t, *J*=7.2 Hz, 4H; -CC**H$_2$**-), 1.24 (t, *J*=7.0 Hz, 12H; -OCH$_2$C**H$_3$**); ^{13}C NMR (90.56 MHz, $CDCl_3$, 25°C): δ=167.2 (-**C**O, esters), 154.5 (-N**C**O-), 135.9, 128.5, 128.3, 128.1 (aromatic carbons), 116.2 (-CN), 67.2 (Ph**C**H$_2$O-), 64.7 (quaternary carbons), 63.0 (-O**C**H$_2$CH$_3$), 47.4 (-N-**C**H$_2$), 30.8 (-**C**CH$_2$-), 13.8 (-OCH$_2$**C**H$_3$); MS: *m*/*z* (%): 735 (100) [*M*+Na]$^+$.

Benzyl 1,1-di(ethoxycarbonyl)-3-(*N*-cyano-*N*-vinylamino)propylcarbamate (13): HPLC analysis R_v=14 mL, Phenomenex, Aqua C_{18}, 250×4.60 mm, 5 μ column, UV_{220}, flow=1 mL min^{-1}, gradient from 50% CH_3OH to 90% CH_3OH in 10 min and then 90% CH_3OH over the next 5 min, 0.1% TFA; ^{1}H NMR (360 MHz; $CDCl_3$): δ=7.34 (s, 5H; aromatic-**H**), 6.24 (s, 1H; -N**H**), 5.88 (dd, 1H; CH_2=C**H**-), 5.10 (s, 2H; PhC**H$_2$**O-), 4.63 (dd, 1H; CH_2=CH-), 4.44 (dd, 1H; CH_2=CH-), 4.21 (m, 4H; -OC**H$_2$**CH$_3$), 3.32 (t, *J*=6.8 Hz, 4H; -N-C**H$_2$**), 2.72 (t, *J*=7.2 Hz, 4H; -CC**H$_2$**-); 1.23 (t, *J*=7.02 Hz, 12H; -OCH$_2$C**H$_3$**); ^{13}C NMR (90.56 MHz; $CDCl_3$): δ=168.6 (-**C**O, esters), 156.1 (-N**C**O-), 137.4 (CH_2=**C**H-), 135.4, 130.0, 129.7, 129.6 (aromatic carbons), 114.1 (-**C**N), 96.7 (CH_2=CH-), 68.8 (Ph**C**H$_2$O-), 66.2 (quaternary carbons), 64.7 (-O**C**H$_2$CH$_3$), 47.8 (-N-**C**H$_2$), 32.7 (-**C**CH$_2$-), 15.3 (-OCH$_2$**C**H$_3$); MS: *m*/*z* (%): 404.3 (25) [*M*+H]$^+$.

2,2,8,8-Tetraethoxycarbonyl-3,4,6,7,8,9-hexahydro-2H-pyrimido[1,2-a]pyrimidine hydrobromide (22): A saturated solution of HCl in ethanol (10 mL) was added to a solution of **12** (10 g, 14 mmol) in absolute ethanol (250 mL). The reaction mixture was stirred overnight at 45°C under a nitrogen atmosphere to give **17** (confirmed by ESIMS). After cooling to RT, 15% Pd/C (500 mg) was added and the reaction mixture was stirred for 2–3 h under an H_2 atmosphere. The mixture was then filtered through a pad of Celite and concentrated to give **19** as a solid residue (confirmed by ESIMS).

Compound **17** was redissolved in absolute ethanol (250 mL) and triethylamine (2.5 mL, 18.2 mmol) was added (**Caution**: Do not use excess base because it leads to the formation of monocyclic guanidinium compound **15**), and the reaction mixture was stirred at 50°C for 2 h. After cooling to RT, the solvent was evaporated in vacuo, and the residue was taken up in CH_2Cl_2, washed with aqueous ammonium bromide solution (3×). The organic layer was dried and concentrated to give slightly brownish solid **22**, which was recrystallized from ethyl acetate/CH_2Cl_2 (9:1) to afford **22** (5.1 g, 74% in four successive steps). HPLC analysis: R_v=20 mL, Phenomenex, Aqua C_{18}, 250×4.60 mm, 5 μ column, UV_{220}, flow=1 mL min^{-1}, gradient from 10% CH_3OH to 50% CH_3OH in 10 min and then to 90% CH_3OH, over the next 10 min, 0.1% TFA; m.p. 136°C (ethyl acetate/CH_2Cl_2); ^{1}H NMR (360 MHz, CD_3CN): δ=9.64 (s, 2H; guanidinium-**H**), 4.26 (q, *J*=7.05 Hz, 8H; -OC**H$_2$**CH$_3$), 3.38 (t, *J*=6.1 Hz, 4H; -N-C**H$_2$**), 2.41 (t, *J*=6.1 Hz, 4H; -CC**H$_2$**-), 1.28 (t, *J*=7.02 Hz, 12H; -OCH$_2$C**H$_3$**); ^{13}C NMR (90.56 MHz, CD_3CN): δ=167.7 (-**C**O, esters), 151.1 (guanidinium carbon), 64.1 (-O**C**H$_2$CH$_3$), 63.1 (quaternary carbons), 44.4 (-N-**C**H$_2$), 26.1 (-C**C**H2-), 14.2 (-OCH$_2$**C**H$_3$); MS: *m*/*z* (%): 428 (100) [*M*+H]$^+$; anal. calcd. (%) for $C_{19}H_{29}N_3O_8$·HBr: C 44.89, H 5.95, N 8.27, Br 15.72; found: C 45.03, H 5.77, N 8.23, Br 15.87.

*Compound **17**: HPLC analysis*: R_v=15 mL, Phenomenex, Aqua C_{18}, 250×4.60 mm, 5 μ column, UV_{220}, flow=1 mL min^{-1}, gradient from 50% CH_3OH to 90% CH_3OH in 10 min and then 90% CH_3OH for a further 5 min, 0.1% TFA; MS: *m*/*z* (%): 759.5 (100) [*M*+H]$^+$.

*Compound **19**: HPLC analysis*: R_v=17 mL, Phenomenex, Aqua C_{18}, 250×4.60 mm, 5 μ column, UV detection at 220 nm, flow=1 mL min^{-1}, gradient from 10% CH_3OH to 50% CH_3OH in 10 min and then to 90% CH_3OH over the next 10 min, 0.1% TFA; MS: *m*/*z* (%): 491.2 (100) [*M*+H]$^+$.

2,2,8,8-Tetra(propylcarbamoyl)-3,4,6,7,8,9-hexahydro-2H-pyrimido[1,2-a]pyrimidine hydrobromide (1): A solution of trimethylaluminium (3.5 mL, 7 mmol, 2M solution in toluene) was added dropwise to a solution of *n*-propylamine (0.493 mL, 6 mmol) in dry CH_2Cl_2 (8 mL) under a nitrogen atmosphere. After stirring at RT for 40 min, a solution of **22** (508 mg, 1 mmol) in CH_2Cl_2 (7 mL) was added dropwise, and the reaction mixture was heated at reflux for 12 h. After cooling, the reaction mixture was quenched by addition of an aqueous HBr solution (47%) and extracted with CH_2Cl_2. The CH_2Cl_2 layer was washed with water, and the aqueous layer was reextracted with CH_2Cl_2. The combined organic layers were dried over magnesium sulfate and concentrated in vacuo to give a white solid that was recrystallized from acetonitrile/ether to afford **1** as white micro crystals (475 mg, 85%). HPLC analysis: R_v=16 mL, Nucleodur-100–5 C_8 ec column, UV_{220}, flow=1 mL min^{-1}, gradient from 10% CH_3OH to 50% CH_3OH in 10 min and then 50% CH_3OH to 90% CH_3OH over the next 10 min, 0.1% TFA; m.p. 222°C (ether/acetonitrile); ^{1}H NMR (360 MHz, CD_3OD, 25°C): δ=8.41 (br s, guanidinium-**H**), 8.27 (t, *J*=5.67 Hz, amide protons), 3.38 (t, *J*=5.6 Hz, 4H; -N-C**H$_2$**), 3.21 (t, *J*=7.03 Hz, 8H; -NC**H$_2$**CH$_2$CH$_3$), 2.47 (t, *J*=6.1 Hz, 4H; -CC**H$_2$**-), 1.55 (h, *J*=7.2 Hz, 8H; -NCH$_2$C**H$_2$**CH$_3$), 0.88 (t, *J*=7.49 Hz, 12H; -NCH$_2$CH$_2$C**H$_3$**); ^{13}C NMR (90.56 MHz, CD_3OD, 25°C): δ=169.2 (-**C**O, amides), 150.8 (guanidinium carbon), 64.9 (-**C**CH$_2$-), 45.5 (-N-**C**H$_2$), 43.0 (-N**C**H$_2$CH$_2$CH$_3$), 28.6 (-C**C**H$_2$-), 23.5 (-NCH$_2$**C**H$_2$CH$_3$), 11.6 (-NCH$_2$CH$_2$**C**H$_3$); MS: *m*/*z* (%): 480.5 (100) [*M*+H]$^+$; anal. calcd. (%) for $C_{23}H_{41}N_7O_4$·HI (as an iodide salt): C 45.47, H 6.97, N 16.14, I 20.89; found: C 45.47, H 6.53, N 15.83, I 19.94; HRMS (microTOF-Q) calcd for $C_{23}H_{42}N_7O_4^+$: 480.3293; found: 480.3289.

2,2,8,8-Tetra(2-methoxyethylcarbamoyl)-3,4,6,7,8,9-hexahydro-2H-pyrimido[1,2-a]pyrimidine hydrochloride (2): A solution of trimethylaluminium (3.5 mL, 7 mmol, 2M solution in toluene) was added dropwise to a solution of 2-methoxyethylamine (0.522 mL, 6 mmol) in dry CH_2Cl_2 (8 mL) under a nitrogen atmosphere. After stirring at RT for 40 min, a solution of **22** (508 mg, 1 mmol) in CH_2Cl_2 (7 mL) was added slowly, and the mixture was heated at reflux for 12 h. After cooling, the reaction was cautiously quenched by adding 6N aqueous HCl. The CH_2Cl_2 layer was washed with water, and the aqueous layer was reextracted with CH_2Cl_2. The combined organic layers were washed with water, brine, and dried with magnesium sulfate. Evaporation of the organic layer left a crude solid that was recrystallized from acetonitrile/ether to yield **2** as a white crystalline solid (435 mg, 75%). HPLC analysis: R_v=10.8 mL, Nucleodur-100–5 C_8 ec column, UV_{220}, flow=1 mL min^{-1}, gradient from 10% CH_3OH to 50% CH_3OH in 10 min and then 50% CH_3OH to 90% CH_3OH over the next 10 min, 0.1% TFA; m.p.: 178–179°C (acetonitrile/ether); ^{1}H NMR (360 MHz, CD_3OD, 25°C): δ=3.35–3.44 (m, 28H), 3.29 (s, 12H; -CH$_2$OC**H$_3$**), 2.41 (t, *J*=5.01 Hz, 4H; -CC**H$_2$**-); ^{13}C NMR (90.56 MHz, CD_3OD, 25°C): δ=169.3 (-**C**O, amide), 151.1 (guanidinium carbon), 71.5 (-CH$_2$**C**H$_2$OCH$_3$), 64.8 (-**C**CH$_2$-), 58.9 (-CH$_2$CH$_2$O**C**H$_3$), 45.4 (-N**C**H$_2$-), 40.9 (-**C**H$_2$CH$_2$OCH$_3$), 29.2 (-C**C**H$_2$-); MS: *m*/*z* (%): 544.5 (100) [M$^+$+H]; anal. calcd. (%) for $C_{23}H_{41}N_7O_8$·HI (as an iodide salt): C

41.14, H 6.30, N 14.60, I 18.90; found: C 41.26, H 6.23, N 14.62, I 18.51; HRMS (microTOF-Q) calcd for $C_{23}H_{42}N_7O_8^+$: 544.3089; found: 544.3095.

2,2,8,8-Tetra(phenylcarbamoyl)-3,4,6,7,8,9-hexahydro-2H-pyrimido[1,2-a]pyrimidine hydroiodide (3): A solution of trimethylaluminium (3.5 mL,7 mmol, 2M solution in toluene) was added dropwise to a solution of aniline (0.546 mL, 6 mmol) in dry CH_2Cl_2 (8 mL) under an inert atmosphere. After stirring at RT for 40 min, a solution of **22** (508 mg, 1 mmol) in CH_2Cl_2 (7 mL) was added dropwise. Then the resulting mixture was heated at reflux for 5 h. After cooling, the reaction was cautiously quenched by addition of aqueous HBr solution (47%). The CH_2Cl_2 layer was washed with water, and the aqueous layer was reextracted with CH_2Cl_2. The combined organic layers were washed with aqueous sodium iodide solution (3×10 mL), dried over magnesium sulfate, and concentrated in vacuo to give a white solid. Recrystallization from acetonitrile/ether afforded **3** as a white crystalline solid (572 mg, 77%). HPLC analysis: R_v=20.2 mL, Nucleodur-100–5 C_8 ec column, UV_{220}, flow=1 mL min^{-1}, gradient from 10% CH_3OH to 50% CH_3OH in 10 min and then 50% CH_3OH to 90% CH_3OH over the next 10 min, 0.1% TFA; m.p. 178–180°C (acetonitrile/ether); ^{1}H NMR (360 MHz; CD_3CN, 25°C): δ=9.19 (s, 4H; amide protons), 8.43 (brs, 2H; guanidinium-**H**), 7.66 (d, J=8.1 Hz, 8H; aromatic), 7.32 (t, J=7.9 Hz, 8H; aromatic), 7.15 (t, J=7.37 Hz, 4H; aromatic), 3.42 (t, J=5.90 Hz, 4H; N-C**H$_2$**-), 2.67 (t, J=6.1 Hz, 4H; -C**H$_2$**-); ^{13}C NMR (90.56 MHz; CD_3CN, 25°C): δ=166.5 (-**C**O, amide), 149.8 (guanidinium carbon), 138.2, 129.7, 126.1, 122.0 (aromatic carbons), 65.4 (-**C**CH$_2$-), 45.5 (-N-**C**H$_2$), 27.9 (-C**C**H$_2$-); MS: m/z (%): 616.4 (100) $[M+H]^+$; HRMS (micrOTOF-Q) calcd for $C_{35}H_{34}N_7O_4^+$: 616.2667; found: 616.2676.

3-(3-Amino-3,3-diethoxycarbonyl)propyl-6,6-diethoxycarbonyl-3,4,5,6-tetrahydropyrimidine (14): 15% Pd/C (150 mg) was added to a solution of **12** (1 g, 1.4 mmol) in absolute ethanol (25 mL). The suspension was stirred in an atmosphere of H_2 for 2 h. The reaction mixture was then filtered through a pad of Celite. The filtrate was evaporated in a vacuum to give a gummy substance that was recrystallized from ether/hexane to afford **14** as a colorless crystalline solid (600 mg, 99%). HPLC analysis: R_v=17 mL, Phenomenex, Aqua C_{18}, 250×4.60 mm, 5 μ column, UV_{220}, flow=1 mL min^{-1}, gradient from 10% CH_3OH to 50% CH_3OH in 10 min and then to 90% CH_3OH over the next 10 min, 0.1% TFA; ^{1}H NMR (250 MHz, $CDCl_3$): δ=7.06 (s, 1H, -C**H**=N-), 4.04–4.15 (m, 8H, -OC**H$_2$**CH$_3$), 3.18 (t, J=7.3 Hz, 2H, -NC**H$_2$**), 3.08 (t, J=5.7 Hz, 2H, -NC**H$_2$**), 2.09 (t, J=6.1 Hz, 2H, -CC**H$_2$**-), 1.99 (t, J=7.1 Hz, 2H, -CC**H$_2$**-), 1.13 (t, J=7.0 Hz, 12H, -OCH$_2$C**H$_3$**); ^{13}C NMR (62.9 MHz, $CDCl_3$): δ=170.50, 169.93 (-**C**O, ester), 150.53 (-**C**H=N-), 64.60, 63.96 (quaternary carbons), 61.86, 61.41 (-O**C**H$_2$CH$_3$), 47.92, 39.66 (-N**C**H$_2$-), 33.70, 25.39 (-C**C**H$_2$-), 13.73, 13.66 (-OCH$_2$**C**H$_3$); MS: m/z (%): 430.4 (100) $[M+H]^+$.

1-[(3-Amino-3,3-diethoxycarbonyl)propyl]-2-amino-4,4-diethoxycarbonyl-1,4,5,6-tetrahydropyrimidine bishydrobromide (15): A solution of 33% HBr in propionic acid (1 mL) was added to a solution of **12** (40 mg, 0.056 mmol) dissolved in dry CH_2Cl_2 (10 mL), and the mixture was stirred at RT for 1 h. The excess HBr was removed by a jet of nitrogen, and the residue was redissolved in acetonitrile (5 mL). The solvent was again removed by a stream of nitrogen to remove residual HBr. The residue was dried under a high vacuum to give **15** as a yellow powder (32 mg, 95%). HPLC analysis: R_v=16 mL, Phenomenex, Aqua C_{18}, 250×4.60 mm, 5 μ column, UV_{220}, flow=1 mL min^{-1}, gradient from 10% CH_3OH to 50% CH_3OH in 10 min and then to 90% CH_3OH over the next 10 min, 0.1% TFA; ^{1}H NMR (250 MHz, CD_3CN): δ=7.96 (s, 1H, guanidinium-**H**); 7.49 (s, 2H, guanidinium-**H**); 4.21–4.38 (m, 8H, -OC**H$_2$**CH$_3$); 3.63–3.70 (m, 2H, -NC**H$_2$**); 3.42–3.51 (m, 2H, -NC**H$_2$**); 2.40–2.51 (m, 4H, -CC**H$_2$**-); 1.22–1.31 (m, 12H, -OCH$_2$C**H$_3$**); ^{13}C NMR (62.9 MHz, CD_3CN): δ=167.64, 165.20 (-**C**O, ester); 153.82 (guanidinium carbon); 65.62 (-O**C**H$_2$CH$_3$), 65.52, (quaternary carbon); 64.85 (-O**C**H$_2$CH$_3$); 63.17 (quaternary carbon); 46.80, 44.14 (-N**C**H$_2$-); 29.58, 26.24 (-C**C**H$_2$-); 14.18, 14.09 (-OCH$_2$**C**H$_3$); MS: m/z (%): 445.3 (100) $[M+H]^+$.

***N,N*-Bis[(3-benzyloxycarbonylamino-3,3-diethoxycarbonyl)propyl]urea (16)**: A 6N aqueous solution of H_2SO_4 (9 mL) was added to a solution of **12** (2.29 g, 3.21 mmol) in CH_3CN (20 mL), and the resulting mixture was stirred in an oil bath at 80°C. After stirring for 30 min, the reaction mixture was cooled to RT, neutralized with 6N aqueous NaOH, concentrated to half its volume, and freeze-dried to give a colorless solid. The solid was taken up in acetonitrile (10 mL), and the insoluble fraction was removed by filtration. The filtrate was then evaporated under vacuo to give urea **16** as a gummy substance (2.3 g, 98%). HPLC analysis: R_v=14 mL, Phenomenex, Aqua C_{18}, 250×4.60 mm, 5 μ column, UV_{220}, flow=1 mL min^{-1}, gradient from 50% CH_3OH to 90% CH_3OH in 10 min and then 90% CH_3OH extended for a further 5 min, 0.1% TFA; ^{1}H NMR (360 MHz, $CDCl_3$): δ=7.25–7.34 (m, 10H, aromatic), 6.29 (s, 2H, -N**H**), 5.74 (brs, 2H, -CON**H$_2$**), 5.07 (s, 4H, PhC**H$_2$**O-), 4.15-4.23 (m, 8H, -OC**H$_2$**CH$_3$), 3.13 (t, J=7.2 Hz, 4H, -NC**H$_2$**), 2.46 (t, J=7.9 Hz, 4H, -CC**H$_2$**-), 1.19 (t, J=7.1 Hz, 12H, -OCH$_2$C**H$_3$**); ^{13}C NMR (62.9 MHz, $CDCl_3$): δ=167.54 (-**C**O, ester), 158.39 (-**C**O-, urethane), 154.73 (-**C**ONH$_2^-$), 135.99, 128.42 128.15, 128.99 (aromatic carbons), 66.99 (Ph**C**H$_2$-), 64.9 (quaternary carbons), 62.83 (-O**C**H$_2$CH$_3$), 42.55 (-N**C**H$_2$-), 31.84 (-C**C**H$_2$-), 13.76 (-OCH$_2$**C**H$_3$); MS: m/z (%): 731.3 (100) $[M+H]^+$.

***N,N*-Bis[(3-amino-3,3-diethoxycarbonyl)propyl]urea (18)**: 15% Pd/C (250 mg) was added to a solution of **16** (3.27 g, 4.47 mmol) in absolute ethanol (35 mL). The obtained suspension was stirred under an atmosphere of H_2 for 3 h. Then the reaction mixture was filtered through a pad of Celite, and the evaporation of the filtrate under vacuo gave **18** as a gummy substance (2.04 g, 99%). HPLC analysis R_v=16 mL, Phenomenex, Aqua C_{18}, 250×4.60 mm, 5 μ column, UV_{220}, flow=1 mL min^{-1}, gradient from 10% CH_3OH to 50% CH_3OH in 10 min and then to 90% CH_3OH over the next 10 min, 0.1% TFA; ^{1}H NMR (250 MHz, CD_3CN): δ=5.35 (brs, 2H, -CON**H$_2$**), 4.14 (q, J=7.0 Hz, 8H, -OC**H$_2$**CH$_3$), 3.20 (t, J=7.3 Hz, 4H, -NC**H$_2$**-), 2.3 (brs, 4H, -N**H$_2$**), 2.08 (t, J=7.3 Hz, 4H, -CC**H$_2$**-), 1.20 (t, J=6.9 Hz, 12H, -OCH$_2$C**H$_3$**); ^{13}C NMR (90.56 MHz, CD_3CN): δ=172.24 (-**C**O, ester), 159.99 (-**C**ONH$_2$), 65.23 (quaternary carbons), 62.78 (-O**C**H$_2$CH$_3$), 42.50 (-N**C**H$_2$-), 33.96 (-C**C**H$_2$-), 14.29 (-OCH$_2$**C**H$_3$); MS: m/z (%): 463.2 (100) $[M+H]^+$.

1-[(3-Amino-3,3-diethoxycarbonyl)propyl]-2-oxo-4,4-diethoxycarbonyl-1,4,5,6-tetrahydropyrimidine (20): A solution of **18** (80 mg, 0.17 mmol) in nitropropane (2 mL) was heated in an oil bath at 135°C for 2 h. After cooling the reaction mixture to RT, the solvent was removed under reduced pressure, and the residue was purified by column chromatography over silica gel (50% ethyl acetate/hexane) to give **20** as a gummy substance (20 mg, 27%). HPLC analysis: R_v=16 mL, Phenomenex, Aqua C_{18}, 250×4.60 mm, 5 μ column, UV_{220}, flow=1 mL min^{-1}, gradient from 10% CH_3OH to 50% CH_3OH in 10 min and then to 90% CH_3OH over the next 10 min, 0.1% TFA; ^{1}H NMR (250 MHz, $CDCl_3$): δ=6.4 (s, 1H, -N**H**-), 5.1 (s, 2H, -N**H$_2$**), 4.2–4.3 (m, 8H, -OC**H$_2$**CH$_3$), 3.7–3.9 (m, 4H, -NC**H$_2$**-), 2.1–2.6 (m, 4H, -CC**H$_2$**-), 1.2–1.3 (m, 12H, -OCH$_2$C**H$_3$**); ^{13}C NMR (62.9 MHz, $CDCl_3$): δ=169.86, (-**C**O, ester), 169.69 (ring -**C**O), 65.29, (quaternary carbons), 62.54 (-O**C**H$_2$CH$_3$), 62.50 (-O**C**H$_2$CH$_3$), 45.14, 39.30 (-N**C**H$_2$-), 31.8, 31.16 (-CCH$_2$-), 13.9 (-OCH$_2$**C**H$_3$); MS: m/z (%): 446.2 (100) $[M+H]^+$

2,2,8,8-Tetracarboxy-3,4,6,7,8,9-hexahydro-2H-pyrimido[1,2-a]pyrimidine hydrobromide (23): A 4N aqueous solution of NaOH (16 mL) was added to a solution of **22** (1 g, 2 mmol) in ethanol (16 mL) and cooled to 0°C. The resulting mixture was stirred at RT for 24 h. The reaction mixture was acidified to pH 1 with 47% HBr solution. Evaporation of the solvent in vacuo left a colorless solid residue that was then taken up in isopropanol (15 mL) and stirred vigorously for 15 min. The insoluble salt residue was removed by filtration, and the filtrate was evaporated in vacuo to give a colorless residue that was recrystallized from acetonitrile/H_2O to afford **23** as a colorless crystalline solid (700 mg, 88%). HPLC analysis: R_v=2 mL, Phenomenex, Aqua C_{18}, 250×4.60 mm, 5 μ column, UV_{220}, flow=1 mL min^{-1}, gradient from 10% CH_3OH to 50% CH_3OH in 10 min and then to 90% CH_3OH over the next 10 min, 0.1% TFA; ^{1}H NMR (250 MHz, D_2O): δ=3.5 (t, J=5.5 Hz, 4H, -NC**H$_2$**-), 2.51 (t, J=5.4 Hz, 4H, -CC**H$_2$**-); ^{13}C NMR (62.9 MHz, D_2O): δ=176.01 (**C**O, acid), 152.10 (guanidinium carbon), 67.23 (quaternary carbons), 47.46 (-N**C**H$_2$-), 29.56 (-C**C**H$_2$-); MS: m/z (%): 228.3 (100) $[(M-2\times CO_2)]^+$, 316.2 (100) $[M+H]^+$.

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[37] a) Crystal structure analysis of **3** (free base): $C_{35}H_{33}N_7O_4$; M_r = 615.68; orthorhombic; space group $P2_12_12_1$ (no. 19); a = 10.2551(5), b = 13.7374(8), c = 21.5253(11) Å; V = 3032.5(3) Å^3; Z = 4; ρ_{calcd} = 1.349 g cm^{-3}; F_{000} = 1296; μ = 0.091 mm^{-1}; R_{int} = 0.1189; 3177 (1899: $I_o > 2\sigma(I_o)$) independent reflections; 419 parameters; $R1$ = 0.0466 ($I_o > 2\sigma(I_o)$); $wR2$ = 0.1237 (all data); GOF = 1.063. The low scattering power of the very tiny crystal forced us to cut the data set at θ = 20.83°. b) Crystal structure analysis of **3** (bromide, acetonitrile adduct): $C_{37}H_{37}BrN_8O_4$; M_r = 737.65; monoclinic; space group $C2/c$ (no. 15); a = 20.0476(1), b = 18.4443(1), c = 20.4080(1) Å; β = 98.2102(2)°; V = 7468.80(7) Å^3; Z = 8; ρ_{calcd} = 1.312 g cm^{-3}; F_{000} = 3056; μ = 1.150 mm^{-1}; R_{int} = 0.043; 6841 (6145: $I_o > 2\sigma(I_o)$) independent reflections; 599 parameters; $R1$ = 0.0269 ($I_o > 2\sigma(I_o)$); $wR2$ = 0.0704 (all data); GOF = 1.057. In addition to the solvent molecules well located in the difference Fourier maps, two unresolved solvent molecules remained and had to be removed by the PLATON calc squeeze procedure. c) Crystal structure analysis of **23** (trihydrate): $C_{11}H_{19}N_3O_{11}$; M_r = 369.29; triclinic; space group $P\bar{1}$ (no. 2); a = 6.5084(1), b = 8.6005(1), c = 14.3244(3) Å; α = 83.9319(5), β = 80.0964(6), γ = 73.5887(10)°; V = 756.30(2) Å^3; Z = 2; ρ_{calcd} = 1.622 g cm^{-3}; F_{000} = 388; μ = 0.147 mm^{-1}; R_{int} = 0.036; 2766 (2494: $I_o > 2\sigma(I_o)$) independent reflections; 303 parameters; $R1$ = 0.0301 ($I_o > 2\sigma(I_o)$); $wR2$ = 0.0792 (all data); GOF = 1.043. d) CCDC 673725 (**3** (free base)), 673724 (**3** (bromide, acetonitrile adduct)), and 673723 (**23** (trihydrate)) contain the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif. For more details, see the Supporting Information.
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